IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: **XUEYING HUANG, *ET AL.***	
APPLICATION NO.: **10/630,248**	GROUP ART UNIT: **1762**
FILED: **JULY 30, 2003**	EXAMINER: **JIMMY LIN**
FOR: **MICROPARTICLE-BASED METHODS AND SYSTEMS AND APPLICATIONS THEREOF**	ATTORNEY DOCKET NO.: **CL 1943 US NA**

DECLARATION UNDER 37 C.F.R. § 1.131

COMMISSIONER FOR PATENTS
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Sir:

1. I Xueying Huang, am a co-inventor with Dr. Ming Zheng [1] in the above-identified patent application.

2. I obtained a Bachelor's degree in Chemistry from Nanjing University, People's Republic of China in 1987 and a Ph.D. in Chemistry from University of Delaware in 1998. I was a post-doctoral fellow at the Stanford University from 1998 to 1999.

3. I am currently employed by Sepax Technologies, Inc., Newark, Delaware, as their Chief Technology Officer.

4. I was an employee of E. I. du Pont de Nemours & Co. Wilmington, DE, United States of America (*hereinafter* "DuPont"), as a Senior Research Chemist, at their

[1] *See* accompanying declaration from Dr. Ming Zheng, also under 37 C.F.R. § 1.131.

Central Research and Development Department (*hereinafter* "CR & D"), from 2000 to 2004.

5. DuPont is the assignee of the present application.

6. While working at C R & D of DuPont, in relation to the above-referenced patent application and otherwise, I made gold nanoparticles coated with ethylene glycol oligomer, and gold nanoparticles coated with a mixture of ethylene glycol and other ligands, that were used by Dr. Ming Zheng to examine protein binding specificities. I prepared said gold nanoparticles in 2001-2002, while working at DuPont CR &D, in Wilmington, DE, USA, at that time. I am no longer employed by DuPont.

7. In the Non-Final Office Action mailed on July 03, 2006, and subsequently in the Non-Final Office Action mailed on June 07, 2007 the Examiner rejected Claims 2, 5-17, and 19 under 35 U.S.C. § 103(a) as being obvious over Templeton, *et al.*, Langmuir 15:66-76 (1999), in view of Foos, *et al.*, Chem. Mater. 14:2401-08 (2002). Specifically, the Examiner asserted that "it would have been obvious to one of ordinary skill in the art at the time of the invention to have used an ethylene glycol oligomer in the preparation of water-soluble gold nanoparticles of Templeton because Foos teaches that an ethylene glycol oligomer can increase the water solubility of a gold nanoparticle."

8. I, Xueying Huang, declare that in September of 2001, at CR & D of DuPont in the United States, Dr. Ming Zheng and I reduced to practice the following entity:

water-soluble, metallic nanoparticles having a mixed monolayer of

(i) a capture-coating component and

(ii) a shielding component;

which was prior to the online publication date of Foos, *et al.* (April 19, 2002). Further to this declaration, I attach notebook pages signed by me and witnessed by co-workers as Exhibits 3H-8H, wherein the dates have been redacted. Also attached are pages from the notebooks of Dr. Ming Zheng, which have been signed by Dr. Ming Zheng and witnessed by his co-workers at DuPont.

Exhibit 1 exemplifies the reduction to practice of water-soluble, metallic nanoparticles made through Ligand Exchange reactions. For example, the data in the lower half of the page show two gel shift assays indicating the absence of non-specific protein binding on to ethylene glycol (abbreviated as "EG-SH" in the notebook page) coated Au particles. A protein called GST (noted by the gene name "pET41a" in the notebook page) was used for the assay. This protein has a GSH binding domain. When mixed with GSH coated Au particles ("Au-GSH"), we observed band shift as shown by lanes "1" in both gel images. When the GSH was exchanged by EG-SH, such band shift disappeared, as shown by lanes "2" in the "4% TBE" gel image, and lanes "2" and "3" the "1% TBE" gel image. These data indicate that EG acts as an efficient shielding component.

Similarly, Exhibit 2 exemplifies the reduction to practice of water-soluble, metallic nanoparticles made through the ligand exchange reactions. Particularly, the data in the upper left gel image show that a mixed monolayer with both EG and GSH at the ratio of 1:6 provides specific binding (band shift with GST protein), yet resists non-specific binding (no band shift with BSA and streptavidin).

Although Exhibits 1 and 2 do not explicitly demonstrate that the final concentration of water in the reaction mixture for the direct synthesis of Au particles with ethylene glycol coating is from about 9% to about 18% V/V, as required by independent Claim 2, as suggested in the "Conclusions" of Exhibit 7H, it is clear that Dr. Ming Zheng and I were cognizant of the importance of the concentration of water to the stability of gold nanoparticles. Particularly, the "Conclusions" (See bottom of notebook Page Exhibit 7H) in Exhibit 7H, which is

dated February 25, 2002, i.e., prior to the effective date of the Foos reference, states that:

1. **Without CH_3COOH, control of $NaBH_4$ could lead to Au~~~EG_4 (a few drops of $NaBH_4$ solution) nanoparticles (purple). It is not stable in H_2O. After days (5~10), some ppt was formed.**

2. **With CH_3COOH, control of $NaBH_4$ is still needed. pH: 2.0→ 5.0? More $NaBH_4$ could be tolerated in the formation of Au~~~EG nanoparticles. Stability?**

The absence of CH_3COOH in the first conclusion (i.e., higher concentration of water) and presence of CH_3COOH in the second conclusion (i.e., lower concentration of water) and my comments about stability indicate my cognizance of the importance of the concentration of water to the stability and yield of the gold nanoparticles with ethylene glycol coating.

The concentration range of 9-18% V/V of water is only a preferred range of water concentration for direct synthesis. Secondly, the approach in Foos relates to ligand exchange. Foos does not relate to the Direct Synthesis Method. Moreover, Foos does not disclose or discuss the water concentration range or its importance in stability of gold nanoparticles. Moreover, Foos does not disclose or discuss the water concentration range or its importance in stability of gold nanoparticles. In fact, Foos relates to ligand exchange reactions, and the water content and its implications are relevant only in direct synthesis method.

The direct synthesis of gold particles coated with ethylene glycol, and ethylene glycol mixed with other ligands was developed and optimized over some period of time, starting no later than November 7, 2001 (see Exhibit 3H, line in the middle of the page), and with first sign of success around February 25, 2002 (See Exhibit 4H, Exhibit 5H TEM image of the $Au(EG)_4$ particle, and Exhibit 6H-8H on exploring conditions for stable particle formation).

9. I, Xueying Huang, also declare that although Exhibits 1, 2, 3H-8H, demonstrate the reduction to practice of the present invention for representative coated metallic nanoparticles, I believe that Dr. Ming Zheng and I have demonstrated reduction to practice for the claimed coated metallic nanoparticles because (i) the binding specificity and (ii) the resistance to non-specific binding are rendered by the choice of ligand and the function of ethylene glycol oligomers. The chemical identity of the core metal does not play a role here because the metal core is buried or shielded by the coating and does not interact directly with the environment.

As a person signing below:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I also declare that all statements were made with knowledge that willful false statements, and the like, are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and any such willful false statements may jeopardize the validity of either the patent application or any patent issuing thereon.

Respectfully Submitted,

9/6/2007

Xueying Huang Date